

# PATENT COOPERATION TREATY

From the  
INTERNATIONAL SEARCHING AUTHORITY

**TRANSLATION**  
**PCT**

WRITTEN OPINION OF THE  
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43bis.1)

To:

Date of mailing  
(day/month/year)

Applicant's or agent's file reference

**PH-2286-PCT**

FOR FURTHER ACTION

See paragraph 2 below

International application No.

**PCT/JP2004/016436**

International filing date (day/month/year)

**05.11.2004**

Priority date (day/month/year)

**05.11.2003**

International Patent Classification (IPC) or both national classification and IPC

Applicant

**NATIONAL INSTITUTE OF ADVANCED INDUSTRIAL SCIENCE AND TECHNOLOGY**

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. FURTHER ACTION

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1bis(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA/JP

Authorized officer

Facsimile No.

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WRITTEN OPINION OF THE  
INTERNATIONAL SEARCHING AUTHORITY

International application No.

PCT/JP2004/016436

Box No. I Basis of this opinion

1. With regard to the language, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ This opinion has been established on the basis of a translation from the original language into the following language  
\_\_\_\_\_, which is the language of a translation furnished for the purposes of international search (under Rule 12.3 and 23.1(b)).

2. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

a. type of material

☒ a sequence listing

☐ table(s) related to the sequence listing

b. format of material

☐ in written format

☒ in computer readable form

c. time of filing/furnishing

☐ contained in the international application as filed.

☒ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

WRITTEN OPINION OF THE  
INTERNATIONAL SEARCHING AUTHORITY

International application No.  
PCT/JP2004/016436

Box No. V	Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement		
<b>1. Statement</b>			
Novelty (N)	Claims	1-9	YES
	Claims	_____	NO
Inventive step (IS)	Claims	_____	YES
	Claims	1-9	NO
Industrial applicability (IA)	Claims	1-9	YES
	Claims	_____	NO
<b>2. Citations and explanations:</b>			
<p>Document 1: C. J. ROSSINI et al., Macromol. Symp. (2001), Vol. 167, pp. 139-151</p> <p>Document 2: A. MANNA et al., World J. Microbiol. Biotechnol. (1999), Vol. 15, No. 6, pp. 705-709</p> <p>Document 3: M. TANSENGCO and I. DOGMA Jr., Acta Biotechnol. (1999), Vol. 19, No. 3, pp. 191-230</p> <p>Document 4: H. J. KIM et al., Antonie van Leeuwenhoek (2003 May), Vol. 83, No. 2, pp. 183-189</p> <p>Document 5: K. SEI et al., Appl. Microbiol. Biotechnol. (2001), Vol. 55, No. 6, pp. 801-806</p> <p>Document 6: JP 10-191980 A (Taisei Corporation), 28 July 1998 &amp; EP 863209 A2 &amp; US 5968801 A</p> <p>Document 7: JP 7-155180 A (Snow Brand Milk Products Co., Ltd.), 20 June 1995 (family: none)</p> <p>Claims 1-9</p> <p>The inventions of Claims 1-9 do not appear to involve an inventive step over documents 1-7 cited in the ISR.</p> <p>Document 1 describes a PHA-decomposing microorganism <i>Streptomyces sp.</i> isolated from a soil sample, and PHA depolymerase isolated and purified from this microorganism, and also describes a method of decomposing PHA using this microorganism or this PHA depolymerase (page 139, Summary, page 142, lines 10-27, page 143, line 10 to page 146, last line). Document 1 also describes, as a method for screening PHA decomposing organisms, a method of culturing microorganisms on agar medium containing PHA and selecting those microorganisms that form clear zones (page 141, line 4 from bottom to page 142, line 9).</p> <p>Document 2 describes 4 strains of genus <i>Streptomyces</i> having the ability to decompose the polyhydroxyalkanoate (PHA), which is one of polyhydroxybutyric acid (PHB), along with a method for decomposing PHB by culturing these strains in PHB solution, and also describes that the ability of the strains to decompose PHB is greater the higher the concentration of PHB in the medium (page 705, Summary, page 706, line 11 from lower left to right column, line 4, page 707, line 4 from lower left to right column, line 3). Document 2 also describes a screening method for PHB-decomposing microorganisms which is similar to that described in document 1 (page 706, lines 20-39).</p> <p>Document 3 describes that 25 strains of actinomycetes having PHB-decomposing ability were obtained by a method of selecting microorganisms which formed clear zones on agar medium containing PHB, and describes a method of decomposing PHB powder using <i>Streptomyces-4</i>, which is one of the 25 strains (page 191, Summary, page 193, Shake Flask Culture, page 194, Clear Zone Test).</p> <p>Document 4 describes the PHA-decomposing microorganism <i>Streptomyces sp.</i> KJ-72 isolated from a soil sample, along with PHA depolymerase isolated and purified from this microorganism, and also describes that this PHA depolymerase exhibits maximum activity at pH 8.7, 50°C, and is highly stable at 40°C or less but loses most of its activity at 60°C (page 183, Abstract, page 186, left column, line 19 to right column, line 3).</p>			

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of: BOX V

Document 5 describes PCR primers and probes for detecting microorganisms having PHB decomposing ability, and describes that these primers and probes were designed based on the homologous regions of 6 types of PHB polymerase, and that one of these 6 types is *Streptomyces exfoliatus* PHB depolymerase (page 801, Abstract, page 803, left column, line 22 to right column, line 4, Fig. 1).

Document 6 describes a polyhydroxyalkanoate-decomposing enzyme derived from a microorganism of the genus *Corynebacterium*, and describes that this enzyme has decomposing activity at a temperature range of 20-60°C with an optimum temperature range of 37-42°C (Claims 1 & 2, paragraph 0023).

Document 7 describes a PHB-decomposing enzyme derived from a microorganism of the genus *Pseudomonas*, and describes that the optimum temperature of this enzyme is approximately 40°C (Claims 1 & 2).

As described in documents 1-3, polyhydroxyalkanoate decomposing enzymes derived from actinomycetes in the genus *Streptomyces*, methods for decomposing PHA using these enzymes, and methods of decomposing PHA using actinomycetes in the genus *Streptomyces* are publicly known, and as described in document 4, *Streptomyces* genus actinomycete-derived PHA depolymerase which functions at high temperatures is also publicly known. Thus, it would be easy to conceive of trying to obtain a PHA-decomposing enzyme derived from another *Streptomyces* genus actinomycete that functions at high temperatures by using the screening methods for PHA-decomposing microorganisms described in cited documents 1-3 or the PCR primers and probes described in documents 5 to isolate a microorganism of the *Streptomyces* genus which decomposes PHA, isolating and purifying a PHA-decomposing enzyme from this microorganism of the *Streptomyces* genus, decomposing PHA by bringing PHA into contact with that PHA-decomposing enzyme, and decomposing PHA by bringing PHA into contact with that microorganism of the *Streptomyces* genus.

Moreover, PHA-decomposing enzymes that function at high temperatures are publicly known in the *Corynebacterium* genus and *Pseudomonas* genus as described in documents 6 and 7; therefore it would be easy to conceive of isolating a microorganism of the *Streptomyces* genus that decomposes PHA in the expectation that microorganisms of the *Streptomyces* genus might also have PHA-decomposing enzymes that function at high temperatures.